

10.5 kb

Book No._____

1/9/95

15/02/2022: To try another miniprep - 10.5 Kb fragment contained in pDELTA1 - and amplify

used overnight cultures grown in the presence of Tef + Kan
saved at 4° , over 60% ind.

alkaline lysis protocol all resuspended in 25 μ l TE
extracted \uparrow from 3 x 1.5 ml culture

Did an enzyme titration } - amplification done only with
+ Mg " } miniprep DNA no plasmids
were used. yet.

included was just 1 R4 with Tag 20

Vol. 50 μ l. 200 μ M dNTP
 4, 3, 2 mM Mg
 4 mM primers
 { 1, 2, 5 U enzyme }
 0.1 0.2 0.5

cycling 3 step 95° 3'
 (95°, 45"
 55°, 30"
 72°, 5') 25 cycles

repared premix with Buffer B containing 2 mM Mg

add supplement Mg accordingly

sed. & mg. unimproved Template
con. limestone.

space 20x matrix

x buffer	100	}	45 * 20	
dATP	20			
P1	4			40
P2	4			5
temp	40			5
enzyme	-	(in 5 µl)		50
H ₂ O	-	(")		

2 mm	3 mm	4 mm
0	5	10
50	45	40
50	50	50

5 μ l PRX as
needed.
enzyme 1 μ l added
5 & 2 17 later
dig. com.

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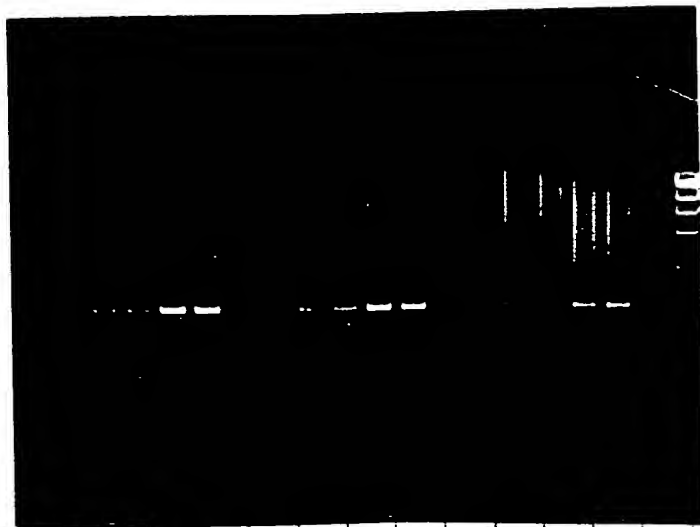
1/10/95

A. Starnam

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1	2		2
3	4	10	3
5	6		4
7	8		2
9	10	20	3
11	12		4
13	14		2
15	16	50	3
17	18		4
19	Tag	20	2 ml

1		2		5				
2	3	4	2	3	4	2	3	4

Result:

cycling has to be optimized. - Lab of mispriming
 2 mM Mg didn't work in any of the sets? - It
 worked earlier in 25 µl 20 µl Tag.

- amount of template?

- Increasing the enzyme didn't seem to work.
 so does Mg

- Tag alone at 20 / 50 µl didn't work

- get fresh enzyme.

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Witnessed & Understood by me,

Date

1/25/95

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Dr. Subramaniam

Date

1/10/95